PATENT
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: James L. Brown
Serial No.: 09/539,735 Group No.: P. Nolan
Filed: 03/30/00 Examiner: 1644
Entitled: **DIAGNOSIS OF AUTOIMMUNE DISEASE**

DECLARATION UNDER 37 C.F.R. § 1.132 BY DR. LEONARD KOHN

Assistant Commissioner for Patents
Washington, D.C. 20231

CERTIFICATE OF MAILING UNDER 37 C.F.R. § 1.8(a)(1)(i)(A)

I hereby certify that this correspondence (along with any referred to as being attached or enclosed) is, on the date shown below, being deposited with the U.S. Postal Service with sufficient postage as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231.

Dated: 12/3/2002 By: _____
Cliff Cannon-Cin

Sir:

1. I, Leonard Kohn, am the subject of the attached Curriculum Vitae (Tab 1) and author of the publications shown on the list attached thereto. On the basis of the information and facts contained in these documents, I submit that I am qualified to speak on the level of ordinary skill in the art of the claimed invention.

2. The Examiner rejected Claims 1, 3-16 and 18 under 35 U.S.C. § 103 as being obvious over Evans *et al.* in view of Yamshiro *et al.*

3. **Difference between the invention and prior art:**

Evans *et al.* disclose the engineered cell line CHO-Rluc. Yamashiro *et al.* show that PEG increases cAMP levels in porcine thyroid cells that are exposed to TS antibody. One difference between Yamashiro *et al.*'s method and the invention's methods is the type of cell used in the assay. Thus, whereas Yamashiro *et al.* uses porcine thyroid cells, the claimed invention uses transgenic hamster ovary cells that have been genetically engineered to express

the human TSH receptor and a luciferase gene under the transcriptional control of a cAMP responsive element.

4. There is no reasonable expectation of success that the recited CHO-Rluc cells will respond to the recited exposure to TS antibody and PEG similarly to the response of Yamashiro *et al.*'s porcine cells to TS antibody and PEG

By way of introduction, the TSH receptor has multiple intracellular signals: it increases arachidonic acid release, and increases inositol phosphate levels, calcium levels and cAMP levels. The effects of these changes on intracellular cell signaling when measuring growth or function is complex and involves both feedback as well as cross talk systems. In general, intracellular signal transduction is cell and stage specific. More specifically, the identity of the intracellular pathway(s) that is signalled by binding of a ligand to the TSH receptor is unpredictable when comparing the same cell type from different animals, the same cell type at different stages of differentiation, and the same cell in response to different ligands that bind to the TSH receptor. Additionally, the mechanism of action of PEG on cAMP is unknown and therefore its effect on cAMP in different cells is unpredictable. Furthermore, even if the same intracellular signalling pathway (*e.g.*, the cAMP pathway) were stimulated in two different cell types, the effect of the increase cAMP levels on cAMP-signalled gene expression in the different cells are unpredictable. These points are further discussed below.

A. Intracellular signal transduction is cell and stage specific

The Examiner found that the presence of the TSH receptor in each of Yamashiro *et al.*'s porcine thyroid cells and in the invention's recited transgenic hamster ovary (CHO-Rluc) cells is predictive of the CHO-Rluc cell's response to TS antibody and PEG. However, it is important to note that the cell's response to ligand binding to the TSH receptor is mediated by **intracellular signal transduction**. In this regard, fully 2 years after filing the instant invention, there remains considerable confusion on the causal effect relationships in intracellular signal transduction. For example, Dumont *et al.* (Tab 2)[1] teach:

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[1] Dumont *et al.* (2002) "Cross signalling, cell specificity, and physiology," AJP 283(1):C2-C28.

> "The literature on intracellular signal transduction presents a confusing picture. . . The confusion of the *in vitro* literature is shown to arise from several causes . . . [one of which is] the implicit assumption that networks of regulation are universal whereas they are in fact **cell and stage specific**."[2]

Therefore, it is a gross oversimplification and a scientific error to assume, as the Examiner does, that responses (such as elevation of cAMP levels and the effect of PEG on these levels) that are mediated by intracellular signal transduction via the TSH receptor would be the **same** in **two different cell types** (*i.e.*, Yamashiro *et al.*'s porcine thyroid cells and the invention's hamster ovary cells).

B. The intracellular signalling pathway(s) induced by binding of a ligand to the TSH receptor is unpredictable in the same cell type from different animals

The cell specificity of intracellular signal transduction is illustrated by the observation that the identity of the intracellular signal that is activated by binding of a ligand to the TSH receptor is unpredictable even when the same cell type from different animal sources is used. For example, Kimura *et al.* (Tab 3)[3] discusses the intracellular cell signalling cascades associated with the TSH receptor in *in vitro* cultured thyroid cells from rat, dog, sheep, and human. Kimura *et al.* teach that the:

> "discrepancies [between the signalling cascades] show that the mechanistic logic of cell cycle stimulation by **cAMP profoundly diverge** in these different *in vitro* models of the **same cell** [*i.e.*, thyroid cell]."[4]

Kimura further underscores the:

> "wide **diversity** of possible mechanisms of cAMP dependent proliferation [and function] in various **cell types**."[5]

Kimura also concludes that:

> "there is no such thing as 'the thyroid cell'. . ."

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[2] (Emphasis added) Dumont *et al.* (2002), Abstract.

[3] Kimura *et al.* (2001) "Regulation of thyroid cell proliferation by TSH and other factors: A critical evaluation of *in vitro* models," Endocrine Review 22(5):631-656.

[4] (Emphasis added) Kimura *et al.*, Abstract.

[5] *Id.*

These teachings emphasize that, even within the same cell type (*i.e.*, thyroid cells), the nature of the intracellular signalling pathway(s) that is initiated downstream of binding to the TSH receptor is unpredictable.

Thus, given Kimura *et al.*'s teachings, one of skill in the art would expect that binding of a ligand to the TSH receptor would result in **divergent** intracellular signalling pathways even in the **same cell type** (*i.e.*, Kimura *et al.*'s thyroid cells) when the cells are obtained from **different animals** (such as Kimura *et al.*'s rat, dog, sheep, and human). Based on this, and taken one step further, the artisan would expect an **even greater divergence** in extrapolating the effect of ligand binding to the TSH receptor when this binding occurs in cells that are of a **different type** (*i.e.*, Yamashiro *et al.*'s **thyroid** cells versus the invention's **ovary** cells) as well as from different animals (*i.e.*, Yamashiro *et al.*'s **porcine** cells versus the invention's **hamster** cells that contain a **human** TSH receptor). This is because the signalling cascades are regulated by the intracellular components that are unique to the cell expressing the TSH receptor, rather than being regulated by the mere presence or absence of a TSH receptor. Moreover, since the identity of the intracellular signalling pathway in the invention's CHO-Rluc cells is unpredictable, it is also unpredictable whether or not, and in what way, the recited PEG would impact this heretofore unknown intracellular signalling pathway.

C. The intracellular signalling pathway induced by binding of a ligand to the TSH receptor is unpredictable in the same cell type at different stages of differentiation

The identity of the intracellular signal that is activated by binding of a ligand to the TSH receptor cannot be extrapolated even at different stages of differentiation in the same cell type. For example, Bell *et al.* (Tab 4)[6] show that binding of TSH to the TSH receptor does **not impact** the cAMP pathway in **preadipocyte cells** while **impacting** the cAMP pathway in **differentiated adipocytes**. In this regard, Bell *et al.* state:

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[6] Bell *et al.* (2002) "TSH signaling and cell survival in 3T3-L1 preadipocytes," AJP 283(4):C1056-C1064.

> "TSH has **no effect** on cAMP levels in 3T3-L1 **preadipocytes** . . . TSH did **increase** cAMP levels fourfold in **differentiated** 3T3-L adipocytes."[7]

Since the identity of the intracellular signalling pathways that is activated by ligand binding to the TSH receptor cannot be predicted even in the same cell type at different stages of differentiation, it is even less predictable which of these multiple intracellular signalling pathways will be activated by binding of a ligand (such as TS antibody) to the TSH receptor in **different cell types** such as Yamashiro *et al.*'s porcine **thyroid** cells *vis a vis* the invention's hamster **ovary** cells. Accordingly, it is even less predictable what impact (if any) PEG would have on this unknown intracellular signalling pathway.

D. The intracellular signalling pathway induced by binding of different ligands to the TSH receptor is unpredictable in the same cell

The complexity and unpredictability of TSH receptor signalling is further underscored by the fact that different ligands that bind to the same TSH receptor in the same cell stimulate divergent intracellular signalling pathways. One need go no farther than Yamashiro *et al.* which state that:

> "the increase in cAMP production by PEG was specific for TSAb as no stimulation was observed by other thyroid simulators such as TSH."[8]

In other words, while each of TSH and TSAb stimulated cAMP levels in Yamashiro *et al.*'s porcine thyroid cells, cAMP levels were enhanced only when TS antibody was the ligand. Importantly, Yamashiro *et al.* does not teach **why** cAMP is elevated when TS antibody, rather than TSH, binds to the TSH receptor in porcine thyroid cells. Nor does Yamashiro *et al.* **explain what factors predict** whether or not cAMP levels will be increased by ligand binding to the TSH receptor. In other words, Yamashiro *et al.* demonstrate that the effect of PEG on cAMP levels can be **diametrically opposite** even in the same cell. This unpredictability is further complicated when one attempts to extrapolate the effect of PEG on cAMP levels in cells that are not only of a different cell type, but also from different animals (*i.e.*, Yamashiro *et al.*'s porcine thyroid cells versus the invention's hamster ovary cells).

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[7] Bell *et al.* page 8, last paragraph.

[8] Yamashiro *et al.*, page 71, last paragraph.

E. The mechanism of action of PEG on cAMP is unknown and therefore its effect on cAMP in different cells is unpredictable

Yamashiro *et al.* expressly state that they **did not know the mechanism** which was responsible for increasing cAMP levels by PEG in response to TS antibody binding to the TSH receptor. They state:

> "the precise mechanism of the stimulating effect of PEG on cAMP production into PTC [*i.e.*, porcine thyroid cells] by TSAb-IgG is *unclear*."[9]

Because the mechanism of action of PEG was unknown, one of skill in the art could not reasonably have predicted whether or not this unknown mechanism also was operative in the recited CHO-Rluc cells, and therefore could not reasonably have predicted whether the recited CHO-Rluc cells would have responded similarly or differently from Yamashiro *et al.*'s cells in the presence of PEG.

F. cAMP-Signalled gene expression is unpredictable in different cells

With respect to Claim 3, even if ligand binding to the TSH receptor induced the same cAMP intracellular signalling pathway in both Yamashiro *et al.*'s porcine cells and the invention's hamster cells (which is unpredictable for the above reasons), the increased cAMP **levels** observed in Yamashiro *et al.*'s cells do not predicate cAMP regulated **gene expression** by observing luciferase activity "using a luminometer" as recited in Claim 3.

For example, Kimura *et al.* (Tab 3) teach that a given stimulus has disparate effects on cAMP regulated **gene expression** in different cell types. Specifically, Kimura *et al.* teach that in the WRT rat thyroid cell line:

> "TSH . . . can independently **activate** Ras and P13K pathways and DNA synthesis [whereas in] dog thyroid primary cultures, TSH (cAMP) does **not activate** Ras and P13K . . ."[10]

Also, increased cAMP **levels** that result from ligand binding to the TSH receptor have different effects on the **expression** of different genes. For example, increased cAMP levels

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[9] (Emphasis added) Yamashiro *et al.*, page 74, second full paragraph.

[10] (Emphasis added) Kimura *et al.*, Abstract.

result in **increased** expression of TG, TPO, and NIS genes [Kohn *et al.* (Tab 5)[11]; Damante & Di Lauro (Tab 6)[12]] but it **decreases** expression of the TSHR gene [Kohn *et al.*, *supra*] and MHC gene [Czech (Tab 7)[13]]. Importantly, the mechanisms that underlie these different effects are unclear [Kohn *et al.*, *supra*; Damante & Di Lauro, *supra*; and Czech, *supra*]. Thus, even if the effect of PEG on cAMP **level** in Yamashiro *et al.*'s porcine cells were erroneously extrapolated to the same effect on cAMP level in the invention's hamster cells, it **cannot be predicted** whether this elevated cAMP level in the invention's hamster cells would result in increased or decreased **gene expression** of the cAMP-signalled reporter luciferase gene, which is the basis for observing luciferase activity "using a luminometer" as recited in Claim 3.

5. In view of the above-discussed **complexity of intracellular signalling** which is **unique** to each **cell type** containing the TSH receptor (whether the TSH receptor is expressed by a wild-type gene as in Yamashiro *et al.*'s cells, or by a transgenic gene as in the recited CHO-Rluc cells), the effects of PEG on increasing cAMP levels in one cell type are **not subject to extrapolation** to an effect on cAMP signal transduction in the same cell type, let alone in another cell type. Therefore, based on my expertise in the relevant art, and in view of the above discussed references as well as the fact that Yamashiro *et al.* uses porcine thyroid cells which are from a **different animal** (porcine versus hamster), **different tissue** (thyroid versus ovary), and which are **not genetically engineered** (versus the genetically engineered CHO-Rluc cells which express the human TSH receptor) in addition to the fact that Yamashiro *et al.* measures a different parameter (cAMP levels versus cAMP-induced gene expression), it is my opinion that one of skill in the art **could not** reasonably predict from Yamashiro *et al.*'s disclosure that the recited CHO-Rluc would be useful in the instantly claimed detection methods. More specifically, it is also my opinion that one of ordinary skill in the art would not have a reasonable expectation of success that the recited CHO-Rluc cells

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[11] Kohn *et al.* (1995) Vitamins and Hormones 50:287-384.

[12] Damante & Di Lauro (1994) Biochem Biophys Acta 218:255-266.

[13] Czech (2000) Cell 100:603-606.

will respond to the recited exposure to TS antibody and PEG similarly to the response of Yamashiro *et al.*'s porcine cells to TS antibody and PEG

6. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing therefrom.

Dated: 11/29/02

By: Dr. Leonard Kohn

Dr. Leonard Kohn